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LETHAL CONCENTRATIONS OF HEAVY METALS IN TISSUE
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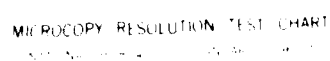
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of the French of Toxicological Research on
LETHAL CONCENTRATIONS OF HEAVY METALS IN TISSUE OF EARTHWORMS

by

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INTRODUCTION

To improve both ecotoxicological test in a chemically defined medium and a procedure of bioavailability study in field, in this first period we worked on

- for ecotoxicological tests : we tried firstly to constitute a breeding stock of earthworms (species Eisenia fetida andrei) in decomposing manure heaps to increase them and make them grow
- for tests : we also installed two chambers with thermostats fixed at 20°C to do toxicity tests and we choose chemical species of heavy metal contaminants
- for field procedure, we improved a practical way to sample both earthworms and soil in very close connections, avoiding artefacts.
- for both field procedure and laboratory tests, we tried to standardize a method to dissolve earthworms in acids and to analyze the concentrations of heavy metals inside
- for field procedure : we started to make a preliminary set of samples in calcareous soils & analysis of them are in progress
- finally, for lab tests, we started the first part of the test (preliminary test) with the different contaminants (Cd, Cu, As, Hg) in an artificial medium named "Artisol".

II. TOXICITY TEST

Method used for the test is based on the European standard for ecotoxicity studies in earthworms (ref; EEC Directive 19/831 Annex V, C, DG XI/129/12, Rev 1-9) (French ref. AFNOR Norme X 31-250).

The test is carried out by two steps. The first one is the preliminary test doses and the second one the definitive test.

We have entirely done the first one and started the second one.

The preliminary test is used for finding the range of concentrations for the definitive test, i.e. the contaminant concentrations causing mortalities of 0 to 100 per cent of earthworms.

2.1. Description of the test method

Adult earthworms species Eisenia fetida are kept in a strictly defined artificial medium called "artisol" which is treated with different concentrations of the test substance. After 14 days, the surviving earthworms at each concentration are counted.

2.1.1. Materials and organisms

a- Materials

The materials include test containers and test substrate.

Test containers used are plastic boxes about 3 liters (21,5 cm x 15 cm x 10,5 cm) with perforated covers.

The test substrate is the medium inside the box where the earthworms are put. We call it "Artisol" and it is composed of two elements:

- a skeleton of glass balls (of about two cm in diameter) : 1425 g of glass balls (+/- one glass ball) per container (about 100 balls per box)

- a matrix which is composed of 90 g of a peculiar silica per test container (trade mark "Levilite") and deionized water : 215 ml per test container with the dissolved test substance in it.

The contaminants are the heavy metals : Cadmium, Copper, Arsenic and Mercury. Thus, test substances will contain these elements. For test substances we have chosen Cadmium chloride 2,5 hydrate ($\text{CdCl}_2 \cdot 5/2 \text{H}_2\text{O}$), Copper (II) chloride dihydrate ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$), Sodium arsenite (NaAsO_2) and Mercury (II) chloride (HgCl_2).

b- Organisms

Test organisms are normally chosen among adults Eisenia fetida, that is to say at least two months old with clitellum, and weight 300-600 mg. They must have about the same size and weight.

2.1.2. Test procedure

For each metal, we have prepared 13 boxes for the test (12 with contaminants and 1 without). Each heavy metal is prepared at three different concentrations. The box without contaminants is the reference or "blank".

Each substance to test is poured in 215 ml deionized water to obtain the correct concentration of heavy metal in each container. When the test substance is well dissolved, the 90 g silica (previously dried at 105°C to be weighted) are added in the solution to make a homogenous matrix. We finally mix the glass balls with the matrix and knead the whole. Thus test containers are ready to receive earthworms.

Before the beginning, those earthworms were gut cleared by placing them during two or three days in "artisol" without contaminant. Then, we wash the earthworms with deionized water and place ten of them in each container onto the medium surface. Boxes are closed with perforated plastic covers and put for 14 days in a chamber at $20^\circ \text{C} \pm 2^\circ \text{C}$ in continuous dark, air humidity 70-90 % RH.

We have prepared three different concentrations for each heavy metal to test. The concentration is expressed as the ratio between the test substance and dry weight silica (at 105°C). The support glass balls are not taken into account in the calculation. The different concentrations are mentioned in the following table.

Metals	Chemicals	Metal molecular weights (g)	Chemical molecular weights (g)	Quantity of chemicals to weight to obtain the following concentrations of the metals in the containers (mg)			
				10 ppm	100 ppm	1000 ppm	10000 ppm
Cu	$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	63,546	170,48	2,40	24,15	241,45	-
As	NaAsO_2	74,922	129,91	1,55	15,60	156,05	-
Hg	HgCl_2	200,59	271,50	1,20	12,20	121,80	-
Cd	$\text{CdCl}_2 \cdot 2,5\text{H}_2\text{O}$	112,40	228,34	-	18,30	182,85	1828,35

2.2. Preliminary results

After 14 days, earthworms are separated from the test medium and washed. We count the worms still alive and the ones already dead.

Results are presented in the following table (percentage of dead earthworms). In the blank we noticed dead earthworms.

CONCENTRATIONS	10 ppm	100 ppm	1000 ppm	10000 ppm
METALS				
Cu	0	70	100	
As	0	0	100	
Hg	0	20	100	
Cd		0	90	100

From results of this preliminary test, we started the second part of the test. This work is in progress.

We choose the ranges corresponding to the concentrations of metals killing between 0 to 100 % of earthworms and we prepared five concentrations in a geometric serie so that the LC50 (concentration of substance which kills 50 % of the test animals during the test period) may be found as exactly as possible.

III. FIELD PROCEDURE

Sampling of some "spots" has been made in the vicinity of Montpellier (France) with the aim to improve practical way to work in field to get a close connection between soil and the various earthworm species sampled. It is necessary to "fix" (prevent any subsequent evolution) soil (by drying) and earthworms (by preservation) in such way to avoid any artefactual contamination. Various solutions are in testing.

To improve our skill to interpret the significance of the so called heavy metal "bioconcentration" of earthworms versus soil properties (incl. soil contamination) we sampled in Spring one first serie of circa 60 "spots" in field and we are making

- zoological analysis of each individual (species, stage, individual weight),
- dissection of them,
- contaminant analysis of soil, body of each earthworm, gut content of each earthworm,
- some physical analysis of soil to know their main properties for further interpretation.

Most analysis are in progress, nevertheless we improve the data management to handle all results (each individual + soil variables) in a data matrix thanks to an improved relational data base (SQL/DS of IBM).

We plan next to make multivariate analysis on such a set of data to improve choices in sampling procedures and soil physical analysis.

The first Spring sampling (in basic, clay, calcareous soil) shall be followed in Fall by one new serie in acid, sandy soils.

After this first transect we plan to compare contaminated and so called non contaminated soils.

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